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ON THE POLARISED FLUORESCENCE—PART DYESTUFFS IN SOLUTION

By KRISHNADAS CHOUDHURI

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ABSTRACT. The variation of the polarisation of fluorescence of the dyestuffs with the wavelengths of the exciting radiation has been investigated in detail. It is found that the polarisation decreases with the increase in wavelength of the exciting radiation, reaches a negative value and then increases again. The negative value occurs at wavelengths of the exciting radiation characteristic for the molecules of the dyestuff. A comparison of the polarisation curves and the absorption curves of the fluorescent dyestuffs has also been made.

The variation of the polarisation of fluorescence of the dyestuffs with the change of (1) the viscosity of the solution, (2) the temperature of the solution and (3) the concentration of the dyestuff has been investigated experimentally. It has been observed that the degree of polarisation tends to vanish asymptotically at low viscosities or at high temperatures or at high concentrations of the dyestuffs, while the polarisation tends to attain asymptotically a certain maximum value at very high viscosities or low temperatures or at very low concentration of the dyestuff. For a particular solvent this maximum value is dependent on (1) the nature of the dyestuff, (2) the wavelength of the exciting radiation. Besides the investigations mentioned above the average life of the dyestuff molecules in the excited state has also been calculated. The results have been discussed on the basis of Perrin's theory.

INTRODUCTION

The phenomenon of fluorescence of the dyestuffs in solution is quite well known and a lot of experimental investigations has been made during recent years. But the results obtained by the various workers are highly contradictory and the discrepancy is so much wild and wide that they can be considered only as giving the order of the magnitude. This is true of all the dominion of fluorescence, including the phenomena of polarisation of the fluorescent radiation emitted by the dyestuffs in solution.

In view of the contradictory nature of the results obtained by the various workers a systematic measurement was undertaken on the polarisation of fluorescence of a few dyestuffs in solution and its exact dependence on the wavelengths of the exciting radiations, on the temperatures, concentration of the dyestuff in solution and the viscosities of the solvent. For our measurements we have selected a set of dyestuffs which has not been systematically investigated as yet. The present report gives the results of our measurement. The results in the case of the inorganic compounds will be given in a subsequent report.

EXPERIMENTAL ARRANGEMENT

The experimental arrangement adopted in our present investigation is as follows :—Light from a mercury arc, automatic zinc or cadmium arcs, rendered monochromatic with the help of a monochromator or suitable colour filters was focused by a lens on a fused quartz cell containing the fluorescent solution. In order to eliminate the uncertain polarisation introduced by the monochromator a polarising prism of Glan type was introduced between the cell and the lens. Thus the fluorescent light was excited by plane polarised monochromatic radiation. The

partial polarisation of the transverse fluorescent light was measured in the usual way with a double image prism and a nicol (Cornu method). Measurements were also made with a Savart plate and an analyser coupled with a pile of glass plates.

As regards the notation adopted by us, the measurements refer to the fluorescent radiation normal to the plane containing the direction of vibration and the direction of propagation of the incident light. The fluorescent light, as already alluded to, is partially polarised, its vibration along with direction of propagation I_2 being usually less intense than that of vibration I_1 perpendicular to the above direction, the percentage of polarisation is given by the relation

$$P=\frac{I_1-I_2}{I_1+I_2}\cdot 100=\rho 100;\ \text{when } I_2>I_1,\ P \text{ is negative.}$$

DEPENDENCE OF POLARISATION OF THE FLUORESCENCE ON THE EXCITING WAVELENGTHS

The fact that the polarisation of the fluorescent radiations emitted by the dyestuffs in viscous solution depends markedly on the wavelength of the exciting radiation was first observed by Frohlick (1925) and nearly a systematic study was first attempted by Wawilow (1929, 1931) who measured the polarisation of a few dyestuffs (Fluorescein, Eosin, Rhodamine) in glycerine solution, when excited by five different wavelengths. The experimental arrangement used by him was to use the light from the monochromator (which was illuminated by a mercury arc) to excite the fluorescence, and measure the polarisation by the Cornu method. Wawilow assumed that the light issuing out of the monochromator, which was used for the excitation of the fluoresecnce is perfectly unpolarised : but it can readily be seen (as was observed by Krishnan and Sirker, 1931) if a measurement be made that the light issuing out of the monochromator is in general considerably polarised,—the actual degree of polarisation differing for different wavelengths. This may be explained as arising out of the selective refraction of the two principal vibrations at the refracting faces of the prism, coupled with a rotation of the plane of polarisation on passing through the quartz parts of the monochromator. On the face of this, the results obtained by Wawilow cease to be of much significance. Later on, Mitra (1939), Jablonski (1934) and Griseback (1936) carried out a systematic investigation on the point.

The results of our measurements of the polarisation of fluorescence excited by different wavelengths are given in the following tables. These tables conclusively show that the polarisation generally decreases to a minimum value as the wavelengths of the exciting radiations decrease and then increases on the further decrease of the wavelengths. This minimum value is negative (*i.e.*, $I_2>I_1$) and is reached when the exciting light is *near about* $\lambda 3131$ A.

Jablonski observed that in the cases of Rose Bengal and other colouring matters in glycerine solutions, the polarisation of the fluorescent radiation progressively decreased with the decrease in wavelengths of the exciting radiation—under the ultraviolet excitation, the polarisation practically vanishes. We are unable to agree with his observation even in the case of Rose Bengal. It seems that Jablonski measured the polarisation of the fluorescent light only for a very few exciting radiations of widely spaced wavelengths and was consequently misled to the wrong conclusions.

It may be mentioned in passing that Griseback observed in the case of eosin that the polarisation attains the maximum negative value at two wavelengths of the exciting radiation instead of at one (312 mμ), which was observed by Wawilow and Mitra. But in all the different dyestuffs that have been investigated, we did not observe any such thing.

TABLE I

Percentage of polarisation of fluorescence of a few dyestuffs in glycerine solution

Temperature :—28°C
Concentration :—c gm/c.c.

Wavelengths in mμ	Succ. Fluorescein c= 5.10⁻⁵	Pinakriptol yellow c= 25 10⁻⁵	Acriflavin c= 8.10⁻³
546	...	36	...
535	...	40	...
517	...	42	...
509	...	43	...
436	45	41	45
405	45	...	44
365	41	38	40
320	0	...	...
313	−10	20	−7
298	−9	−8	...
278	6	0	...
265	20	...	...
254	31	12	36

TABLE I—*contd.*

Percentage of Polarisation of a few dyestuffs in glycerine solution

Wavelengths in mμ	Pinaflavol c= 05.10⁻⁵	Rose Bengal c= 5 10⁻⁵	Succ. Eosin c=4.10⁻⁵	Erythrosin c=4.10⁻⁵
546	46	42	39	50
535	...	...	43	50
509	..	...	..	49
436	36	38	45	45
405	..	..	..	41
365	26	27	27	24
326		...	−8	8
313	14	−9	−9	−10
298	−8	...	−7	−5
278	−8	...	...	...
254	20	37	36	35

POLARISED FLUORESCENCE IN OTHER SOLVENTS

We have also measured the polarisation of the fluorescent radiation emitted by these dyestuffs in other solvents. The solvents used were castor oil, collodion-ether mixture, sugar solution, etc. The Tables and curves show beyond doubt that the value of "P" is different for different solvents, the general nature of the graphs practically remains the same as in glycerine.

TABLE II

Percentage of Polarisation of Fluorescence in Castor oil

Concentration :—c gm/c.c.
Temperature :—28°C

Wavelength in mμ	Succ. Fluorescein c= 5.10⁻⁵	Succ. Eosin c= 4.10⁻⁵	Acriflavin c= 8.10⁻⁵	Pinaflavol c= 05.10⁻⁵
546	...	35	...	42
436	42	40	42	32
365	38	25	35	24
326	0	−10	...	..
313	−12	−12	−8	10
278	5	...	...	−9
254	25	30	32	20

TABLE II

Percentage of Polarisation of Fluorescence in collodion-ether mixture

Concentration :—c gm/c.c.
Temperature :—28°C

Wavelength in mμ	Succ. Fluorescein c= 5.10⁻⁵	Succ. Eosin c=4.10⁻⁵	Acriflavin c= 8.10⁻⁵	Pinaflavol c= 05.10⁻⁵
546	...	40	...	45
436	45	43	42	38
365	40	29	40	25
326	0	−5	...	...
313	−12	−12	−8	12
278	...	...	...	−6
254	20	30	33	21

TABLE III

Percentage of Polarisation of Fluorescence in sugar solution

Concentration of the compounds :—c gm/c.c.
Temperature :—28°C

Wavelength in mμ	Succ. Fluorescein c= 5.10⁻⁵	Succ. Eosin c=4.10⁻⁵	Acriflavin c= 8.10⁻⁵	Pinaflavol c= 05.10⁻⁵
546	...	42	...	41
436	43	40	41	36
405	41	...	...	...
365	35	25	36	26
326	0	−8	...	...
313	−10	−10	−9	8
278	6	...	...	−7
254	21	27	30	23

ULTRAVIOLET ABSORPTION OF THE DYESTUFF AND THE NEGATIVE POLARISATION

The observations of Griseback and Mitra reveal the resemblance of the absorption and the polarisation curves of the fluorescent dyestuffs. Griseback found in the case of fluorescein, eosin and rhodamine B that the maximum negative value for the polarisation was obtained when the dyestuff is excited by radiation of wavelength which corresponds to the junction of the two absorption bands in the ultraviolet. On the other hand Mitra observed the same in the case of fluorescein alone but not in the case of eosin or rhodamine B.

However, as the ultraviolet absorption data are now at our disposal (Chowdhury and Bose, 1943) we can gauge how far contentions of the aforesaid workers are true. In the accompanying graphs are drawn the absorption coefficient curves as well as the polarisation curves superimposed on the same graph :—

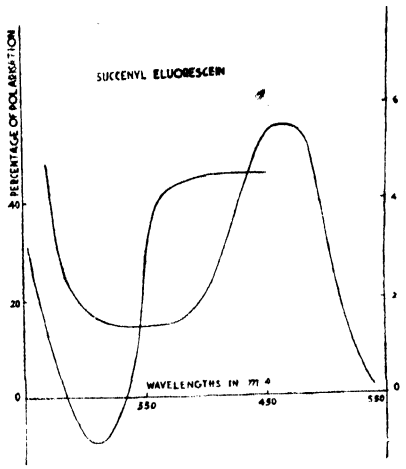

FIG 1

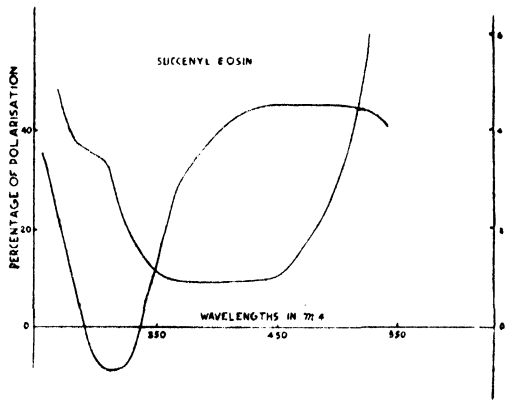

FIG 2

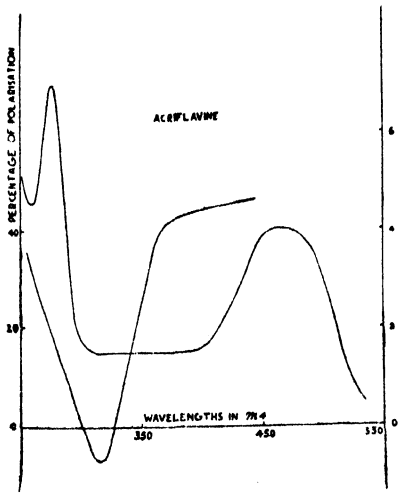

FIG. 3

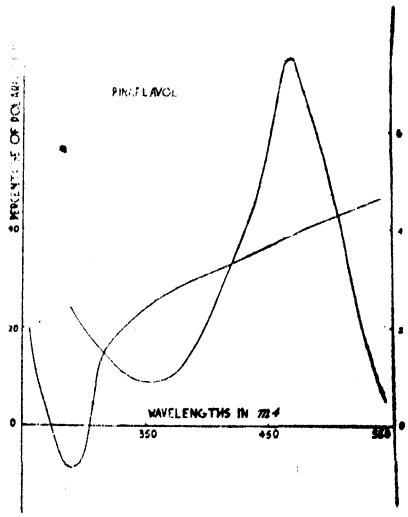

FIG. 4

Fig. 1, 2, 3, 4 show absorption and polarisation curves of Succ. Fluorescein, Succ. Eosin, Pinaflavol, and Acriflavin.

INFLUENCE OF THE VISCOSITY OF THE SOLUTION OF THE POLARISATION

We have already alluded to the fact that the polarisation of the fluorescence depends on the viscosity of the fluorescing solution. This has been experimentally demonstrated by the investigations of Pringsheim (1924), Perrin (1929), Wawilow (1922), Mitra (1938, 1939) and others.

It is first Perrin who gave a satisfactory theory of the dependence of the polarisation on the viscosity of the fluorescing solution, and deduced on the basis of the theory of Brownian rotation of the molecules an expression for the above dependence. The principle of his theory is as follows:—Assuming that the molecules are rigidly fixed in space, if an incident polarised light excites fluorescence in the medium, the polarisation of the fluorescent radiations will have a certain value p_0 which is characteristic of the substance. If, on the other hand, as in actual experiments, the molecules are rotating, the mean square of the angle of rotation per second can be calculated in terms of the viscosity of the solution and its temperature from Einstein's theory of Brownian motion; hence the "expectation" of rotation for a time τ can be calculated, where τ is the mean duration of the molecules in the excited state. The smaller this angle of rotation, the more closely would the actually observed value of the polarisation p approach the ideal value p_0. Thus for very large viscosity p should reach asymptotically the limiting value p_0, while at low viscosities the polarisation ought to reach again asymptotically zero value.

TABLE IV

Polarisation of Acriflavin in glycerine-water mixtures. Exciting wavelength :—λ 4358 A

Percentage by wt. of glycerine	Viscosity in poises	Percentage of Polarisation	
		$c=3.10^{-5}$ gm/c.c.	$c=80.10^{-5}$ gm/c.c.
98	4.56	44	45
90.9	1.65	38	42
83.3	.611	30	34
76.9	.367	23	28
71.4	.185	17	22
66.6	.127	12	17
62.5	.097	8	12
58.5	.070	5	9
51.2		almost zero	5

TABLE V

Percentage of Polarisation of Acriflavin in glycerine-water mixtures. Exciting wavelength :—λ 3650 A

Percentage by wt. of glycerine	Viscosity in poises	Percentage of Polarisation	
		$c=3.10^{-5}$ gm/c.c.	$c=8.10^{-5}$ gm/c.c.
98	4.56	40	42
90.9	1.65	34	38
83.3	.611	25	30
76.9	.367	18	23
71.4	.187	11	15
66.6	.127	6	9
62.5	.97	—	5

We shall quote here only the final expression obtained by Perrin, *viz.*,

$$\frac{1}{p}=\frac{1}{p_0}+\left[\frac{1}{p_0}-\frac{1}{3}\right]\frac{R\theta}{V\eta}\tau.$$

where p is the degree of polarisation observed under the actual condition of the experiment and p_0 the value for the same molecules when they are not allowed to rotate from their initial

orientations. R is the gas constant, V, the gram-molecular volume, η the co-efficient of viscosity and θ the absolute temperature and τ is the mean duration of the fluorescing molecules in the excited state.

TABLE VI

Percentage of Polarisation of Erytherosine in glycerine-water mixtures.

Exciting wavelength:— λ 4358 A
Concentration :— 2.10^{-5}gm/c.c.

Percentage by wt. of glycerine.	Viscosity in poises.	Percentage of Polarisation.
98	4.56	35
90.9	1.65	27
83.3	.611	20
76.9	.367	14
71.4	.185	9
66.5	.127	5

TABLE VII

Percentage of Polarisation of Rose Bengal in glycerine-water mixtures.

Exciting wavelength :— λ 4358 A
Concentration :— $1'2.10^{-5}$gm/c.c.

Percentage by wt. of glycerine.	Viscosity in poises	Percentage of Polarisation.
98	4.56	35
90.9	1.65	26
83.3	.611	18
76.9	.367	13
71.4	.185	7
66.5	.127	3

TABLE VIII

Percentage of Polarisation of Succ. Fluorescein in glycerine-water mixtures.

Exciting wavelength;— λ 4358 A

Percentage by wt. of glycerine.	Viscosity in poises.	Percentage of Polarisation	
		$c = '05.10^{-5}$ gm/c.c.	$c = '5.10^{-5}$ gm/c.c.
98	4.56	48	45
90.9	1.65	44	40
83.3	.611	36	31
76.9	.367	29	25
71.4	.185	24	19
66.6	.127	17	11
62.5	.097	12	5

TABLE IX

Percentage of Polarisation of Succ. Fluorescein in glycerine-water mixtures.

Exciting wavelength :— λ 3650 A.

Percentage by wt. of glycerine.	Viscosity in poises.	Percentage of Polarisation	
		$c = '05.10^{-5}$ gm/c.c.	$c = '5.10^{-5}$ gm/c.c.
98	4.56	40	38
90.9	1.65	36	34
83.3	.611	30	26
76.9	.367	25	20
71.4	.185	19	13
66.6	.127	14	8
62.5	.097	9	3

TABLE X

Percentage of Polarisation of Pinaflavol in glycerine-water mixtures.

Exciting wavelength:—λ4358A. Concentration:—$'05.10^{-5}$gm/c.c.

Percentage by wt. of glycerine.	Viscosity in poises	Percentage of Polarisation.
98	4.56	38
90.9	1.65	32
83.3	.611	24
76.9	.367	18
71.4	.185	12
62.5	.097	4
58.3	.070	Zero

TABLE XI

Percentage of Polarisation of Pinakriptol yellow in glycerine-water mixtures.

Exciting wavelength :— λ 4358 A. Concentration:—$'25.10^{-5}$gm/c.c.

Percentage by wt. of glycerine.	Viscosity in poises	Percentage of Polarisation
98.	4.56	38
90.9	1.65	27
83.3	.611	17
76.9	.367	12
66.6	.127	5
58.8	.070	0

In order to be able to have some information regarding this duration of the molecules in the excited states or the average life of the fluorescing molecules, as they are sometimes called, measurements were made on the polarisation of the fluorescence of the dyestuffs under investigation in glycerine-water mixtures, whose relative proportions and hence the viscosity could be varied over a wide range. The concentration of the dyestuffs per c.c. of the solution was kept constant in all the cases, so also the temperature of the solution.

The values of the viscosity were calculated from the extensive measurements as given by Shelly (1932) on viscosity at different temperatures and various concentrations by graphical interpolation.

The results of our measurements are shown in the accompanying tables. It will be observed that in all cases the degree of polarisation tends at high viscosity to reach asymptotically the maximum limiting value p_0, whereas at low viscosity the polarisation tends towards zero value. This limiting value does not only depends on the nature of the dyestuff but also on the wavelength of the exciting radiation.

The mean life of the fluorescing molecules in the excited states τ has also been calculated, the following gives the results of our calculation. It is evident from the nature of the expression for τ that for values of p very near p_0, as also very near zero, the calculated value of τ cannot be reliable. Hence τ has been calculated only for the values of $p > \cdot 10 < \cdot 45$.

TABLE XII

Average life τ of Acriflavin in glycerine water mixture.

Viscosity in poises	Excitation by λ 4358		Excitation by λ 3650	
	$c = 3.10^{-5}$ gm/c.c.	$c = \cdot 8.10^{-5}$ gm/c.c.	$c = 3.10^{-5}$ gm/c.c.	$c = \cdot 8.10^{-5}$ gm/c.c.
1.65	$1 \cdot 2.10^{-8}$ Sec.	$7 \cdot 5.10^{-9}$ Sec.		
.611	$1 \cdot 1.10^{-8}$ Sec.	$6 \cdot 9.10^{-9}$ Sec.	$1 \cdot 0.10^{-8}$ Sec.	$6 \cdot 0.10^{-9}$ Sec.
.367	$1 \cdot 3.10^{-8}$ Sec.	$7 \cdot 1.10^{-9}$ Sec.	$1 \cdot 1.10^{-8}$ Sec.	$7 \cdot 0.10^{-9}$ Sec.
.185	$1 \cdot 2.10^{-8}$ Sec.	$7 \cdot 4.10^{-9}$ Sec.	$1 \cdot 2.10^{-8}$ Sec.	$7 \cdot 7.10^{-9}$ Sec.

TABLE XIII

Average life τ of Sacc. Fluorescein in glycerine water mixture.

Viscosity in poises	Excitation by λ 4358		Excitation by λ 3650	
	$c = \cdot 5.10^{-5}$ gm/c.c.	$c = \cdot 05\ 10^{-5}$ gm/c.c.	$c = \cdot 5.10^{-5}$ gm/c.c.	$c = \cdot 05.10^{-5}$ gm/c.c.
1.65	$10 \cdot 6.10^{-9}$ Sec.	$6 \cdot 0.10^{-9}$ Sec.	$9 \cdot 7.10^{-9}$ Sec.	$6 \cdot 8.10^{-9}$ Sec.
.611	$9 \cdot 7.10^{-9}$ Sec.	$6 \cdot 1.10^{-9}$ Sec.	$9 \cdot 4.19^{-9}$ Sec.	$6 \cdot 1.10^{-9}$ Sec.
.367	$9 \cdot 7.10^{-9}$ Sec.	$6 \cdot 8.10^{-9}$ Sec.	$10 \cdot 1.10^{-9}$ Sec.	$6 \cdot 2.10^{-9}$ Sec.
.185	10.10^{-9} Sec.	$5 \cdot 2.10^{-9}$ Sec.	$10 \cdot 3.10^{-9}$ Sec.	$5 \cdot 6.10^{-9}$ Sec

TABLE XIV

"Average life" of Pinaflavol in glycerine-water mixtures.

Exciting wavelengths = λ 4358 A. Concentration = $\cdot 05.10^{-5}$ gm/c.c.

Viscosity in poises =	4.56	1.65	.116	.673	.518
$\frac{(3-p_0).R\theta}{V}.\tau$	1.44	1.54	1.37	1.46	1.38

It is evident from the tables that within the limits of the experimental errors the values of τ is practically independent of the viscosity (provided it is low), though the values are widely different for the different concentrations of the dyestuffs. Moreover, the values of the average life are independent of the exciting wavelengths.

We have already reported the phenomena of quenching of fluorescence by foreign neutral salts. We can consider this act of quenching as something like a quantum mechanical resonance, if we look at it in the light of collision process of the second kind. If now on undergoing different excitations with different wavelengths, the excited molecules had different probabilities of quenching, some dependence of the quenching on the wavelength of exciting light should be observed. But experimentally it was shown by Wawilow (1929) Sveshnikow

(1935) and Mitra (1938), that there is no dependence of the quenching action on the exciting wavelengths. Their conclusions show that the excited molecules have all the same energy level during the excited state. Thus our conclusions arrived at from the polarisation measurements are in agreement with those of the aforesaid investigators.

INFLUENCE OF TEMPERATURE ON THE POLARISATION OF THE FLUORESCENT RADIATIONS

As regards the influence of temperature on the polarisation of fluorescence Pringsheim and Perrin observed that with increase of temperature, the polarisation gradually dwindles down, finally vanishing to zero. Mitra, over and above this observed that with the decrease of temperature, the polarisation asymptotically tends towards the maximum value " p."

We have also measured the polarisation of the dye-stuffs under investigation in glycerine solution at various temperatures of the solvent. The results of our measurements are given below.

We can readily understand that the influence of temperature on the polarisation will be two-fold ; directly owing to the greater thermal agitations at higher temperatures the " expectations " of rotation of the molecules after excitation from their initial positions will be greater and hence the value of the polarisation will correspondingly be smaller. It also affects indirectly by changing the viscosity of the solution. Both the effects work in the same direction; *viz.*, to diminish the polarisation at higher temperatures. Both these effects have been taken into consideration in the already alluded Perrin's theory. If now these are the only effects of temperature, the average life calculated on the basis of the aforesaid theory ought to be independent of the temperatures. Such an independence was shown by Mitra to be experimentally true only in the case of dilute solution of the dyestuff ; but in the case of concentrated solutions the life-time of the molecules gradually falls down with the increase of temperature, showing thereby that there are other effects of temperature besides the two already mentioned.

Experimentally the value of the polarisation was measured for the temperatures from 0° to about 90°C.

The results of our measurements are given in the following Tables:—

TABLE XV

Influence of temperature on the polarisation of fluorescence of Acriflavin in glycerine excited by λ 4358

Temperature in °C	Percentage of Polarisation		
	$c=3.10^{-5}$ gm/c.c.	$c=\cdot 8\ 10^{-5}$ gm/c.c.	$c=\cdot 1.10^{-5}$ gm/c c.
10	50	50	50
20	48	50	50
30	44	46	50
40	38	41	44
50	33	36	39
60	27	31	34
70	21	25	29
80	16	20	23
90	10	15	18

TABLE XVI

Influence of temperature on the polarisation of fluorescence of Acriflavin in glycerine excited by λ 3650

Temperature in °C	Percentage of Polarisation		
	$c=3.10^{-5}$ gm/c.c.	$c=\cdot 8.10^{-7}$ gm/c.c.	$c=\cdot 1.10^{-5}$ gm/c.c.
0	42	42	42
10	42	42	42
20	41	42	42
30	37	38	40
40	32	34	36
50	27	29	31
60	22	24	26
70	16	18	20
80	10	12	15
90	4	7	10

TABLE XVII

Influence of temperature on the polarisation of fluorescence of Pinaflavol and Rose Bengal in glycerine excited by $\lambda 4358$

Concentration: 1×10^{-5} gm/c.c.

Temperature in °C	Percentage of Polarisation	
	Pinaflavol	Rose Bengal
0	42	45
10	42	44
20	42	41
30	41	35
40	36	29
50	28	22
60	22	15
70	15	8
80	8	—
90	—	—

TABLE XVIII

Influence of temperature on the polarisation of fluorescence of Pinakriptal in glycerine

Concentration—$\cdot 1.19^{-5}$gm/c.c.

Temperature in °C	Percentage of Polarisation excited by λ	
	4358	3650
0	45	42
10	45	42
20	41	36
30	36	32
40	30	25
50	24	18
60	16	12
70	10	6
80	5	

TABLE XIX

Influence of temperature on the polarisation of fluorescence of Succ. Eosin in glycerine

Concentration = $\cdot 2.10^{-5}$gm/cc.

Temperature in °C	Percentage of Polarisation excited by λ	
	4358	3650
0	45	42
8	45	42
20	43	39
30	39	35
40	35	28
50	29	23
60	24	17
70	19	11
80	13	6

The general nature of the curves is the same as that representing the variation of p with the viscosity. At low temperatures p tends to reach asymptotically the limiting value p_0 as for large viscosities, while at high temperatures p tends to vanish. On calculating the value of τ on the basis of the Perrin's theory we get the following results:—

TABLE XX

Influence of temperature on the average life of the Acriflavin molecules in the excited state. Exciting wavelength $-\lambda 4358$

Temperature in °C	Average life of the molecules		
	$c = 3.19^{-5}$ gm./c.c.	$c = \cdot 8.10^{-5}$ gm./c.c.	$c = \cdot 1.10^{-5}$ gm./c.c.
30	13.10^{-9} sec.	8.10^{-9} sec.	—
40	13.10^{-9} ,,	9.10^{-9} ,,	6.10^{-9} sec.
50	11.10^{-9} ,,	8.10^{-9} ,,	6.10^{-9} ,,
60	13.10^{-9} ,,	9.10^{-9} ,,	7.10^{-9} ,,

TABLE XXI

Influence of temperature on the average life of the Acriflavin molecules in the excited state, Exciting wavelength $-\lambda 3650$

Temperature in °C	Average life of the molecules		
	$c = 3.10^{-5}$ gm/c.c.	$c = \cdot 8.16^{-5}$ gm./c.c.	$c = \cdot 1.10^{-5}$ gm./c.c.
30	13.10^{-9} sec.	10.10^{-9} sec.	—
40	13.10^{-9} ,,	10.10^{-9} ,,	7.10^{-9} sec.
50	11.10^{-9} ,,	9.10^{-9} ,,	7.10^{-9} ,,
60	13.10^{-9} ,,	10.10^{-9} ,,	7.10^{-9} ,,

These results reveal that though the value shows a very striking dependence on the concentration of the dyestuff, it is practically independent of the temperature within the ranges of concentrations we have studied. This shows conclusively that only effects of raising the temperature are through increased thermal agitation and the consequent larger value of expectation of the Brownian rotation of the dyestuff molecule per second and through diminished viscosity.

DEPENDENCE OF THE POLARISATION ON THE CONCENTRATION OF THE DYESTUFF IN SOLUTION

We have also seen from the foregoing tables that the polarisation of fluorescence exhibits a conspicuous change with the change of the concentration of the fluorescing dyestuffs.

The dependence was first experimentally shown by Pringsheim, who observed that as the concentration is increased the polarisation decreases. This observation was afterwards confirmed by Gaviola, Wawilow and others. Mitra carried out a detailed investigation and found that with the increase of concentration polarisation tends to vanish while at very small concentration it tends to reach asymptotically the same limiting value as is reached at large viscosities and low temperatures.

Our data also reveal that with the increase of concentration the values of the polarisation tend to vanish; while at very small dilutions, it tends to reach asymptotically the same limiting value as is reached at large viscosities or low temperatures.

ON THE MAXIMUM VALUE OF THE POLARISATION OF THE FLUORESCENCE OF THE DYESTUFFS

It has already been referred that the limiting value of the polarisation is its value when the molecules after excitation are not allowed to rotate from their initial orientations, which is evidently the value of the polarisation when the viscosity is very large or temperatures very low. The following table exhibits the values of this limiting polarisation for the various dyestuffs in glycerine solution when excited by radiation of various wavelengths as were obtained in our present investigation.

TABLE XXII

Dyestuffs in glycerine	Excitation wavelength in A°	Maximum limiting value of polarisation
Acriflavin	4358	·50
	3650	·42
Pinakriptol	4358	·45
	3650	·42
Succ. Eosin	4358	·45
	3650	·42
Rose Bengal	4358	·45
Pinaflavol	4358	·42

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PHYSICS LABORATORY,
DACCA UNIVERSITY.

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